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HERPETOMONAS MUSCAE-DOMESTICAE, ITS BEHAVIOR AND EFFECT IN LABORATORY ANIMALS

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While making some observations on the habits of blood sucking flies, at a large dairy during the summer of 1921, the writer noticed that many of the house flies frequenting cattle were engorged with blood. A microscopc examination of the alimentary tract proved that the blood was obtained from cows. Closer observations revealed the fact that house flies frequently feed at the punctures recently deserted by *Stomoxys calcitrans* or *Haematobia serrata.* It was noticed that house flies hovered near stable or horn flies when feeding and immediately pounced upon the wounds inflicted by these insects as soon as the biting flies had flown away to rest and digest their meals. The house flies in turn sucked blood until engorged. These observations demonstrated certain possibilities under which house flies might act as transmitters of microörganisms and parasites found at times within the peripheral circulation.

Darling in 1912 proved that *Musca domestica* was able to transmit *Trypanosoma hippicum* to healthy mules after feeding on the sores of diseased ones.

Patton and Cragg in 1913 showed that some non-biting members of the genus Musca, like *M. pattoni,* associated themselves around cattle with such biting forms as Haematopota, Stomoxys, Philaematomyia, and other forms. Most of the non-biting species feed only on blood and depend entirely on the biting species for the preparation of their food. Patton and Cragg further noticed that the non-biters were not house flies, but species which are seldom found away from cattle. In the Philippines in 1912, Mitzmain observed a close association between non-biting flies and Stomoxys, but the non-biting species were not identified.

Since no protozoa were at this time found in biting flies, and since the house fly offered possibilities for the dissemination of blood parasites, it became important to make a careful study of the protozoan

fauna found in the alimentary tract of such flies; to attempt to cultivate these protozoa, and determine their pathogenic properties. Three species of flagellates were found in the digestive tract of house flies during the summer of 1921, but only one of these will receive consideration at this time.

Herpetomonas muscae-domesticae Burnett was by far the most prevalent flagellate inhabiting the digestive tract of adult house flies. When the Herpetomonad was present at all, it was nearly always there in enormous numbers; sometimes the entire intestinal contents seemed to consist of little else. In order to avoid falling into an error, the writer considered only those flies parasitised which showed the true flagellated form. Possible preflagellate or postflagellate types of the parasite were not tabulated for the reason that some of these are easily confused with certain stages of the two other species obtained and since cultivated by me in pure culture.

Dissections of house flies from June 1 until the middle of November gave the following percentages of infected individuals.

	Number dissected	Infection
June	80	12.5 per cent.
July	110	41.0 per cent.
August	125	52.0 per cent.
September	95	21.0 per cent.
October	50	4.0 per cent.
November	50	0 per cent.

Judging from the writings of others, the percentages of infected flies found here in New Jersey correspond more nearly to the percentages obtained by workers in warmer climates. In Madras, Patton found 100 per cent. infected. In Syria, Wenyon states that the majority of the house flies harbored *H. muscae-domesticae*. Prowazek found 8 per cent. infection at Rovigno, while Franchini and Mantovani found a 3 per cent. infection near Boulogne. Dunkerly and Hewitt were unable to find the parasite in England and the writer knows of no records in America.

Franchini and Mantovani (1915) discovered their infected flies on farms in the environment of Boulogne. Flies caught in the houses of Boulogne showed no Herpetomonads. This observation was independently verified by the writer during the summer. In going over his notes he does not find a single record of a dissection of a fly caught within dwelling houses that revealed any flagellates whatever. All of the parasitised flies were caught in cow barns and stables. This seems to suggest a rather close association between the Herpetomonad and domestic animals.

The flagellate under discussion received much attention by other workers on account of its similarity to the parasite of Kala Azar. Prowazek (1904), Patton (1908-1909), Porter (1909), MacKinnon (1910), Wenyon (1911 and 1913) fully described the life history and cytology of the flagellate from films and sections of the intestines of house flies. During all of this time much discussion arose concerning various morphological details. Prowazek, Lingard and Jennings, claimed that the Herpetomonad had a double flagellum, while Léger, Patton, Porter, MacKinnon, and Wenyon insisted that the animal had a single flagellum and that a double flagellum represents the beginning of longitudinal division of the parasite.

The present writer obtained some excellent films from flies fixed and stained with Giemsa which seem to prove that the flagellum is single and that the so-called "double nature" of the flagellum is a division phenomenon. Figures 1, 2, 3 and 4, show various types of the mature and maturing Herpetomonad. The trophonucleus is situated near the center of the lancet shaped body. The blepharoplast is located at the anterior flagellar end. Figure 5 represents the flagellum in the act of splitting longitudinally accompanied or preceded by the division of the blepharoplast. Figure 6 shows a further advance consisting in the division of the trophonucleus accompanied or succeeded by cell division. A separation of the two halves of the flagellum is noticed at the anterior end of the protozoan. Figure 7 is the last stage preceding the separation of the two daughter halves. Figure 3 shows a deeply staining thread which runs backward from the kinetonucleus. That this thread is spirally coiled as claimed by Prowazek could not be determined. In the writer's specimens the thread appears nearly straight. Some individuals were found which showed a distinct vacuole at the extreme anterior end (Fig. 3). Figures 8 and 9 represent other morphological forms of the same species of Herpetomonad frequently encountered. A cytopharynx as found by Wenyon (1913) was not observed. Moreover, whole or even parts of bacteria are not found within the endoplasm. It is highly probable that the protozoan is nourished through osmotic diffusion currents.

Since the morphology and life history of the flagellate has been completely worked out by others, the writer attempted very little in this direction. In anticipation of experimental work, however, it was thought important to identify the species here in New Jersey with the European form. Notwithstanding various difficulties encountered in interpreting conflicting statements in the literature concerning the morphology of *Herpetomonas muscae-domesticae* (often also referred to as *Leptomonas muscae-domesticae* or *Crithidia muscae-domesticae),* the writer is fairly certain that he is dealing if not with the identical species, at least with a variety very closely related to the form or forms

studied by Prowazek, Patton, Wenyon, Franchini, Mantovani, and other European and Asiatic workers.

In 1915, a fresh impetus was imparted to the whole Herpetomonad work by Laveran and Franchini. These investigators found a Herpetomonad in the mouse flea resembling *H. muscae-domesticae* in many respects. They were able to cultivate the flagellate in pure culture on the Nicolle, Novy and McNeal medium. Laveran and Franchini found Leishmaniform bodies in their cultures after a few days which later developed into true Herpetomonads. Later, in 1919, these same observers produced a typical form of Leishmaniasis in white mice by inoculating them with cultures of the Herpetomonad found in the intestine of the mouse flea. Rats also proved susceptible and developed Leishmaniasis, better called Herpetomaniasis as suggested by Fantham and Porter (1916). The disease was also reproduced by feeding the parasites. The Herpetomonads of *Anopheles maculipennis* and *Melophagus ovinus* produced similar conditions. In 1919, Chatton also cultivated a Herpeomonad from the dog flea and reproduced Leishmaniasis in dogs.

In all of these experimental cases, the animals were inoculated or fed with the flagellated forms found in culture. After periods varying between six weeks and two and a half months, the animals began to show symptoms such as fever, emaciation, with loss of appetite and weight. Often Leishmaniform and sometimes flagellated forms were found in the blood. Autopsies of very sick animals, and those that died of the disease usually showed an enlargement of the spleen in which the rounded or oval Leishmaniform bodies were found. These bodies also proved quite numerous in the bone marrow. The liver rarely revealed any parasites and the other organs never.

In 1916, Fantham and Porter reported a large number of experiments with Herpetomonads and Crithidia obtained from the digestive tract of a variety of insects. With these flagellates they were able to produce Herpetomoniasis in mice, dogs, canaries, sparrows, martins, grass snakes, lizards, frogs, toads, and sticklebacks. From the experiments the authors concluded that:

"Herpetomoniasis or Leishmaniases can be induced in various warm and cold blooded vertebrates when the latter are inoculated or fed with Herpetomonads occurring in the digestive tracts of various insects. The infection produced and the protozoan parasites found in the vertebrates resemble those of human and canine Leishmaniases." "The disease induced may run an acute or a chronic course. In the acute cases among the vertebrates, the flagellate form of the parasite was the more obvious at death. In chronic cases, non-flagellate forms of the parasite were more numerous."

Fantham and Porter argue that since the flagellated stages of *Leishmania donovani* and *L. tropica* are now known, the links com-

pleting the evidence that a Leishmania is a Herpetomonas are complete. They further express the belief "that Leishmaniases are invertebrate-borne Herpetomoniases, and that these maladies have been evolved from flagellates of invertebrates (especially Herpetomonads of insects) which have been able to adapt themselves to life in vertebrates."

In 1915 Franchini and Mantovani stated that they were able to produce Leishmaniasis or Herpetomoniasis in rats with *H. muscae-domesticae,* the house fly parasite. Besides reproducing the usual symptoms of Herpetomoniasis and the Leishmaniform stages in the involved organs, they were further able to cultivate the organism indirectly. House flies in contradistinction to fleas and some other insects have a prolific intestinal flora which makes it impossible to obtain pure cultures directly from any part of the alimentary canal. The present writer has many times convinced himself of the fruitlessness of such an attempt. The bacteria carried over into the media together with the *Herpetomonads* soon outgrow them and in a short time kill off the flagellates. These protozoa seem to be able to tolerate a certain amount of intestinal flora under intestinal conditions, but soon die out under artificial conditions, i. e., on artificial media upon which bacteria multiply so rapidly.

Franchini and Mantovani took blood from the heart of one of their rats inoculated three and one-half months previously with the intestinal contents of parasitised house flies. Some of this blood was inoculated into the condensation liquid of the N.N.N. medium (1 part) mixed with a 3 per cent. soluton of glucose (4 parts). In about twelve days a pure culture of little organisms appeared having the aspect of anaplasms in stained smears. At autopsy two mice inoculated intraperitoneally with this culture showed a few Leishmaniform parasites in the liver. The authors were unable to propagate their cultures in transplants.

The present writer inoculated intraperitoneally four white and two wild mice, one rat, and one guinea pig with the intestinal contents of flies heavily parasitised with *Herpetomonas muscae-domesticae.* Care was taken to obtain parasites in the flagellated condition and they seemed active and vigorous. The inoculated animals showed no clinical symptoms whatever. Their blood was examined in stained and unstained condition at intervals of a few days, but nothing was found. Two of the white mice were autopsied in one month. All of the organs were normal and no Leishmaniform bodies were visible in the liver, spleen, bone marrow, kidneys, or other organs. One white mouse and one wild mouse were killed after two months. The examinations were entirely negative. One white mouse, one wild mouse, and the rat were sacrificed in three months. The white mouse had pneumonic lungs, but in every other respect the three animals were normal. No parasites were found. Since the examinations of the supposedly susceptible

animals proved negative, and since the guinea pig showed no symptoms nor parasites in the blood, it was not killed and is still alive today, five months after the inoculation. At no time during this period did any temperature develop nor was there any loss in weight.

The negative results obtained by the writer need not reflect on any of the results obtained by Franchini and Mantovani, and suggest that the authors may have dealt with a geographical variety or with a distinct species, although for morphological reasons the European form and the form studied from this locality may be considered identical. It may be suggested that the *Herpetomonads* of house flies in different parts of the world be carefully compared, and also that a careful seasonal study of the forms occurring in one region be made.

Since it seemed impossible to obtain a pure culture of *H. muscae-domesticae* free from bacteria indirectly by the inoculation of higher animals, another method that proved successful was devised. In 1918, while experimenting with grasshopper diseases and strains of d'Herelle's *Coccobacillus acridiorum,** it was found that many species of Acridians developed an immunity toward bacteria. Many Acridians die of bacterial infections annually, but many also recover and become sexually mature. Such recovered hoppers are comparatively immune and this immunity can be demonstrated. Experimentally also, it was shown that this immunity could be produced in healthy non-exposed animals by inoculation with sublethal doses or with killed cultures of various bacteria. The idea suggested itself that it might be possible to inoculate grasshoppers or Locustids with the intestinal contents of flies containing Herpetomonads and bacteria, and perhaps obtain a pure culture of the protozoa in this way.

In the first experiment, fifty large female grasshoppers *(Melanoplus femur-rubrum)* were inoculated with such material. On August 3 the intestines of five heavily parasitised house flies were removed under aseptic conditions and cut up very finely in sterile Locke's solution in order to liberate the Herpetomonads and mince the intestine, so that no large pieces of tissue would be introduced into the hoppers. The hoppers were then held and restrained, so that the inoculation site could be wiped off with alcohol corrosive sublimate mixture. Twenty-five hoppers were then inoculated each with 0.1 c.c. of Locke's solution containing Herpetomonads, bacteria, intestinal cells and contents. Ten hoppers were inoculated into the body cavity on the ventral side between the thorax and abdomen, and fifteen were inoculated in the hind leg joint between the trochanter and femur. Twenty-five uninoculated hoppers were kept as controls. The inoculated and uninoculated hoppers were then placed into separate sterile glass jars with some grass.

* A systematic study of the organisms distributed under the name of *Coccobacillus acridiorum.* d'Herelle. Annals Ent. Soc. America, 1918, vol. xi.

In 48 hours all but four of the inoculated hoppers were dead. The uninoculated ones were all alive. The dead hoppers gave off an odor of putrefaction and were swarming with bacteria. No Herpetomonads were seen. The four inoculated, but live hoppers were also carefully examined. The leg joint between the trochanter and femur of the hind leg was first wiped off with alcohol and then singed. A sharp, sterile capillary pipette was then introduced and some blood removed. Some of this blood was introduced into ordinary culture media to test for bacterial sterility. The rest was examined microscopically for bacteria and Herpetomonads. In none of the four hoppers were bacteria found microscopically, but in three of the animals a few actively moving Herpetomonads were seen. These preparations were stained by Giemsa's method and the flagellates were identical with those introduced. The N.N.N. medium and a variety of other media were inoculated with some of the blood containing Herpetomonads, but no growth was obtained at room or incubator temperature although tubes were kept for two weeks and examined every few days. Some tubes were also sealed in order to produce a lowered oxygen tension. The media previously inoculated with blood to prove bacterial sterility remained sterile.

Blood from the control hoppers was carefully examined for flagellates in exactly the same manner. No bacteria nor protozoa of any sort were found. In the writer's previous experiences with hundreds of hoppers comprising many species, he has never found protozoa in the blood of these animals.

These experiments, therefore, prove that out of a large series of hoppers inoculated with the intestinal contents of parasitised house flies, a small number will survive, will free themselves of the intestinal bacteria in about 48 hours, and will maintain *Herpetomonas muscae-domesticae* for at least 48 hours or more.

Since no growth of Herpetomonads was obtained on the media used, an insect medium suggested itself, but since one can secure so little blood serum or tissue juices from hoppers another insect was used.

The writer was fortunately rearing large numbers of the larvae of the meal moth *(Euphestia küniella)*. About two hundred large meal worms were taken and rubbed up in a mortar until nearly all of the juices were expressed. Twenty-five c.c. of sterile Locke's solution was added at intervals to facilitate the grinding. This material was then strained through a cheese cloth after which it was filtered through paper. The filtrate amounted to 35 c.c. This was then filtered through sterile Berkefeld candles. At the same time some fresh horse serum was filtered through a Berkefeld candle. To 10 c.c. of the diluted insect juices, 30 c.c. of horse serum was added and the two materials mixed. This mixture was then tubed into small tubes, 1.5 c.c. per

tube. The tubes were then put into an inspissator and the temperature raised to 74 C., at which temperature the serum coagulated. The time during which the temperature was raised from 60 C. to 74 C., and lowered again to 60 C. consumed one hour and thirty minutes. The tubes were then removed. Two drops of normal grasshopper blood were then permitted to flow over each slant, after which the tubes were incubated for 48 hours as a test for bacterial sterility. Later the tubes were stored in the refrigerator after being sealed with sealing wax to prevent evaporation.

August 18, fifteen grasshoppers *(Melanoplus femur-rubrum)* and two locustids *(Amblycorypha oblongifolia)* were again inoculated as in the previous experiment. Fifteen hoppers constituted the controls. In 48 hours all the controls were alive excepting two. These two were examined but nothing excepting bacteria found. All the experimental hoppers but one were dead. The two locustids were alive. Blood was examined from the hopper and two locustids and Herpetomonads were found in all three. No bacteria were present, nor did any growth appear subsequently in inoculated media. Some blood from the three positive cases was inoculated into some of the special insect-horse serum medium previously described. Two tubes were inoculated from each animal, sealed with sealing wax and kept at room temperature. At the end of seven days the tubes were examined and a light growth of Herpetomonads was found in the liquid in the bottom of some of the tubes. The growth was found in one tube inoculated with hopper blood and in two tubes inoculated from the blood of one of the locustids. The two tubes inoculated with blood from the other locustid showed no growth. All the tubes were sterile for bacteria. In the tubes in which growth occurred the Herpetomonads were all in the flagellated condition growing in rather dense clumps. No pre- or post-flagellated phases were seen and those present, as stained smears demonstrated, appeared to have been produced by the longitudinal division of pre-existing flagellated forms. When the flagellates were examined in Locke's solution they appeared to be quite vigorous and active, although their activity was somewhat hampered by excessively long and wavy flagella. The flagella at times appeared to be twice the length of the flagella seen in preparations of the Herpetomonads direct from fly intestines. Otherwise, the morphology of the cultivated forms corresponded to the long, lancet shaped forms found in flies. Transfers to fresh insect media were immediately made. In the original tubes the flagellates died out during the course of the next week. After a week the transfers again showed a growth, but not nearly so prolific as in the original tubes. Some material from one of these tubes was inoculated intraperitoneally into two white mice. One mouse was autopsied in four weeks and the other in ten weeks. Absolutely nothing was found.

A second cultural transfer was made, but nothing grew. Examinations every few days revealed a partial recovery of the flagellates put in, but these soon died out and no multiplication occurred. The organisms in the first transfer tubes also died out very soon.

While the examinations were being made, 15 hoppers were inoculated September 5. The results were almost identical with the foregoing. A fairly luxuriant growth was obtained on the first set of tubes. Transfers were made and the resulting growth was weaker. Second transfers were again attempted, but no growth ensued.

SUMMARY

House flies in proximity to cattle were found engorged with cow blood. A close association between the feeding habits of Stomoxys, Hæmatobia and *Musca domestica* was observed. House flies often feed at the punctures deserted by the biting flies. *Herpetomonas muscae-domesticae* was found to be the most prevalent flagellate inhabiting the digestive tract of adult house flies in summer. The number of flies parasitised was large. The greatest degree of parasitism was reached in July and August. The parasitised flies were always caught in cow barns and in horse stables. Flies caught in dwelling houses were not parasitised.

Some morphological details pertaining to the flagellate are discussed and the opinion is expressed that the morphology described is identical with that studied by other workers.

Experimental Leishmaniases or Herpetomoniasis is reviewed and discussed. The writer was unable to produce either with *Herpetomonas muscae-domesticae.*

The view is tendered that probably more than one variety of *Herpetomonas muscae-domesticae* exist and that these varieties may be detected solely on the basis of pathogenic and other physiologic properties.

A special method for the pure cultivation of *Herpetomonas muscae-domesticae* is described. The flagellated form was cultivated and reproduced itself by longitudinal division.

REFERENCES CITED

Darling, S. T., 1912.—Experimental infection of the mule with *Trypanosoma hippicum* by means of *Musca domestica.* Jour. Exp. Med., 15: 365.

Dunkerly, T. S., 1911.—On some stages in the life history of *Leptomonas muscae-domesticae* with some remarks on the relationships of the flagellate parasites of insects. Quart. Jour. Micr. Sci., 56: 645.

Franchini, G., and Mantovani, M., 1915.—Infection expérimentale du rat et de la souris par "*Herpetomonas muscae domesticae.*" Bull. d. la Soc. d. Path. Exotique, Tome 8, No. 3: 109.

Fantham, H. B., and Porter, A., 1916.—The significance of certain natural flagellates of insects in the evolution of disease in vertebrates. Jour. Parasitology, 2: 149.

Léger, L., 1902.—Sur la structure et le mode de multiplication des Flagelles du genre Herpetomonas, Kent. C. R. Ac. Sci., 134: 781.

Laveran, A. et Franchini, G., 1919.—Infection des souris blanches à l'aide des cultures de Herpetomonas ctenocephali. Bull. d. la Soc. d. Path. Exotique, 12: 379.

MacKinnon, D. L., 1910.—Herpetomonads from the alimentary tract of certain dung-flies. Parasitology, 3: 255.

Mitzmain, M. B., 1912.—The rôle of Stomoxys calcitrans in the transmission of *Trypanosoma evansi*. Phil. Jour. Sci., Sect. B., 7: 475.

Porter, A., 1909.—The life-cycle of *Herpetomonas jaculum* (Léger) parasitic in the alimentary tract of Nepa cinerea. Parasitology, 2: 367.

Prowazek, S., 1904.—Die Entwicklung von Herpetomonas, einem mit Trypanosomen verwandten Flagellaten. Arb. aus dem Kaiserl. Gesundheitsamte, 20: 440.

Wenyon, C. M., 1913.—Observations on *Herpetomonas muscae-domesticae* and some allied flagellates with special reference to the structure of their nuclei. Arch. f. Protistenk., 31: 1.

Explanation of Plate XI

All figures represent *Herpetomonas muscae-domesticae*. × 813.

Figs. 1-4.—Types of mature and maturing flagellates.

Fig. 3.—Herpetomonad showing deeply stained rod, and vacuole at anterior end.

Fig. 5.—Division of flagellum and blepharoplast.

Fig. 6.—Division of trophonucleus and cell.

Fig. 7.—Advanced cell division.

Figs. 8 and 9.—Other morphological types.

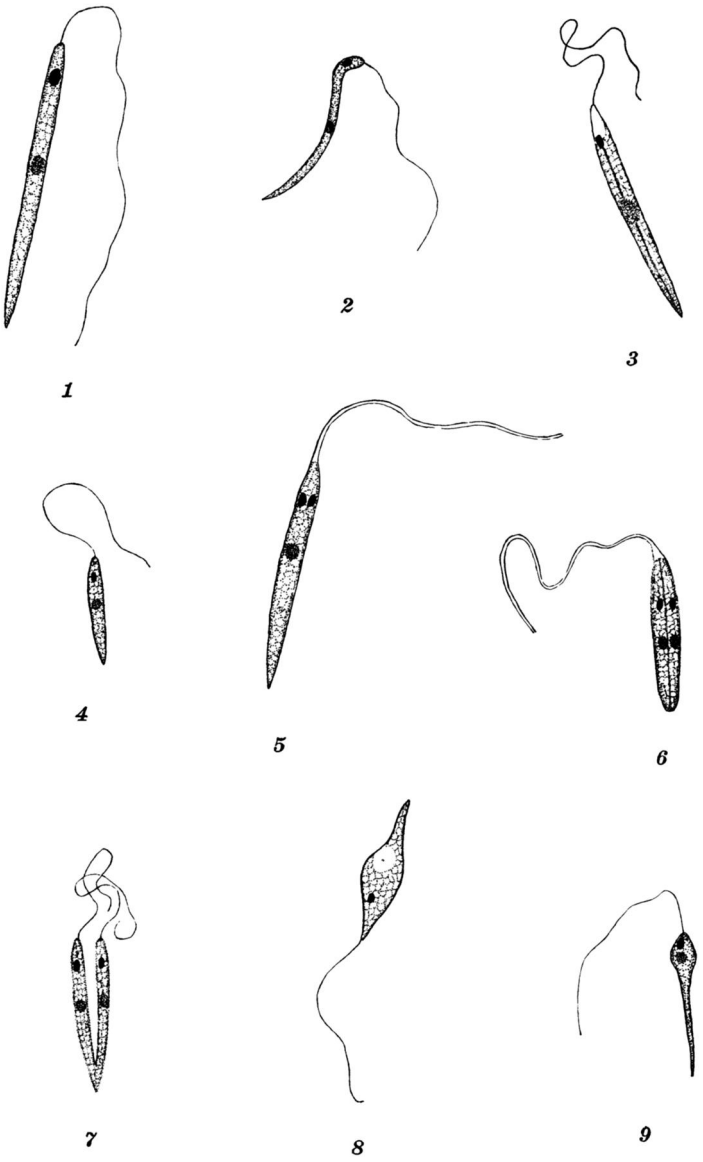

PLATE XI